D E C L A R A T I O N

In the matter of U.S. Patent Application Ser. No. 10/070,387 in the name of Naoki MIDOH, et al.

I, Kyoko IMAMURA, of Kyowa Patent and Law Office, 2-3, Marunouchi 3-Chome, Chiyoda-Ku, Tokyo-To, Japan, declare and say:

that I am thoroughly conversant with both the Japanese and English languages; and,

that the attached document represents a true English translation of Japanese Patent Application No. 1999-253040 filed on September 7, 1999.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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[TITLE OF INVENTION] CYCLIC DEPSIPEPTIDE SYNTHETASE GENE

[SCOPE OF CLAIMS]

[Claim 1]

A gene carrying the following DNA (a) or (b):

(a) DNA of a nucleotide sequence shown as SQ ID No. 1;

(b) a microorganism-derived DNA hybridizing with the DNA of the nucleotide sequence (a) under stringent conditions and encoding a protein with cyclic depsipeptide synthetase activity.

[Claim 2]

A protein described below in (a) or (b):

(a) a protein of an amino acid sequence shown as SQ ID No. 2; or

(b) a protein of an amino acid sequence prepared through deletion, substitution or addition of one or several amino acids in the amino acid sequence (a) and with cyclic depsipeptide synthetase activity.

[Claim 3]

A recombinant vector carrying a gene according to claim 1.

[Claim 4]

A microorganism harboring a recombinant vector according to claim 3 and expressing the gene.

[Claim 5]

A microorganism according to claim 4, wherein the microorganism is a fungus producing the substance PF1022.

[Claim 6]

A method for producing the substance PF1022 and a derivative thereof by using a microorganism according to claim 4 or 5.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to the cyclic depsipeptide synthetase gene for producing the substance PF1022 as a cyclic depsipeptide with anthelmintic activity, a protein encoded by the gene, a recombinant vector using the gene, and a method for producing the substance PF1022 in a substance PF1022-producing microorganism integrated with the recombinant vector.

[0002]

[Prior Art]

The substance PF1022 [cyclo(D-lactyl-L-N-methylleucyl-D-3-phenyllactyl-L-N-methylleucyl-D-lactyl-L-N-methylleucyl-D-3-phenyllactyl-L-N-methylleucyl)] is the cyclic depsipeptide produced by a filamentous fungus of Agonomycetales, namely the strain PF1022 [Mycelia sterilia; the strain was deposited as FERM BP-2671 at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan]. The substance PF1022 exerts an extremely high anthelmintic activity on nematodes parasitic on animals [Japanese Patent publication No. 35796/1991; Sasaki, T. et al., J. Antibiotics., 45, 692 (1992)]. Therefore, the substance is useful as an anthelmintic for animals and is additionally useful as a raw material for the synthesis of a more highly active derivative of the substance.

[0003]

The amounts of secondary metabolites produced by microorganisms and separated from natural origins are generally very slight. So as to industrially utilize such metabolites, therefore, the productivity of the secondary metabolites is necessarily increased. For the increase of the productivity, the examination of the culturing methods and the culture medium

components, the modification of the fermentation conditions such as addition of the precursors, and the mutation of the strains by ultraviolet irradiation or mutagen are carried out. In recent years, the productivity has been increased by utilizing gene recombinant technology in addition to the aforementioned methods.

[0004]

As the method, the enhancement of the expression of the genes of enzymes involved in the biosynthetic pathways, the enhancement of the expression of the genes involved in the control of the biosynthesis, and blockage of unnecessary biosynthetic pathways and the like are practically effected [Khetan, A. and Hu, W.-S. Manual of Industrial Microbiology and Biotechnology 2nd edition, p. 717, (1999)]. As a specific example, further, it has been known a method for improving the productivity, comprising allowing the hemoglobin gene of a bacterium to be expressed for the purpose of the improvement of the oxygen usability [Minas, W. et al., Biotechnol. Prog. 14, 561, (1998)].

[0005]

The most general means for the improvement of the productivity by gene recombinant technology is the enhancement of the expression of the genes of enzymes involved in biosynthetic pathway. For the application of the means, essentially, the transformation method of a subject microorganism is established; a promoter and a terminator applicable to the enhancement of the expression are to be present; and the biosynthetic pathway is to be elucidated and their genes are to be isolated. The transformation method of a fungus producing the substance PF1022, comprising integrating a foreign gene, has already been established (WO 97/00944); and also, a report concerning the promoter and terminator

applicable to the enhancement of the expression has been issued (Japanese Patent Application No. 252851/1999). However, no gene involved in the biosynthetic pathway has been isolated.

[0006]

The substance PF1022 is of a structure comprising L-N-methylleucine, D-lactic acid, and D-phenyllactic acid, which are bonded together through ester bonds and amide bonds. It is considered that the substance is synthesized from 4 molecules of L-leucine, 2 molecules of D-lactic acid and 2 molecules of D-phenyllactic acid by a certain peptide synthetase in a fungus producing the substance. Peptide synthetase means an enzyme involved in the biosynthesis of microbial secondary metabolites, such as peptide, depsipeptide, lipopeptide and peptide lactone, from substrates of amino acids and hydroxy acid. The sequences of some peptide synthetases have already been elucidated [Marahiel, M. A. et al., Chem. Rev., 97, 2651, (1997)]. The reaction with such enzyme is totally different from the synthetic system of protein with ribosome using mRNA as template. Peptide synthetase has one domain for each substrate, where each substrate is activated with ATP for binding through phosphopantothenic acid in the domain; and the resulting bound substrates form amide bonds or ester bonds due to the catalytic actions in the regions between the individual domains.

[0007]

[Problem to be solved by the invention]

The cyclic depsipeptide synthetase gene capable of improving the productivity of the substance PF1022 is provided, by permitting excess expression thereof in a fungus producing the substance PF1022.

[0008]

[Means for Solving the Problem]

So as to overcome the problem, the inventors isolated the cyclic depsipeptide synthetase gene for the synthesis of the substance PF1022 from a fungus producing the substance PF1022, on the basis of the sequence of the conserved region of a known peptide synthetase. Furthermore, the inventors successfully improved the productivity of the substance PF1022 by preparing an expression vector modified with the promoter and terminator of the gene and integrating the expression vector in the fungus producing the substance PF1022. Thus, the invention has been achieved.

[0009]

More specifically, the invention relates to the following aspects.

1. A gene carrying the following DNA (a) or (b):

(a) DNA of a nucleotide sequence shown as SQ ID No. 1; or

(b) a microorganism-derived DNA hybridizing with the DNA of the nucleotide sequence (a) under stringent conditions and encoding a protein with cyclic depsipeptide synthetase activity.

2. A protein described below in (a) or (b):

(a) a protein of an amino acid sequence shown as SQ ID No. 2; or

(b) a protein of an amino acid sequence prepared by deletion, substitution or addition of one or several amino acids in the amino acid sequence (a) and with cyclic depsipeptide synthetase activity.

3. A recombinant vector carrying a gene in a first aspect of the invention.

4. A microorganism harboring a recombinant vector in a third aspect of the invention and expressing the gene.

5. A microorganism in a fourth aspect of the invention, wherein the microorganism is a fungus producing the substance PF1022.

6. A method for producing the substance PF1022 and a derivative thereof by using a microorganism in a fourth or fifth aspect of the invention.

[0010]

[Mode for Carrying out the Invention]

The cyclic depsipeptide synthetase gene of the invention can be isolated from a fungus producing the substance PF1022 for example by the following method.

[0011]

A library comprising the genomic DNA of a fungus producing the substance PF1022 is prepared by extracting the genomic DNA from the fungus producing the substance PF1022, cleaving the DNA with an appropriate restriction endonuclease, and subsequently ligating to a phage vector.

Based on the conserved region of the amino acid sequence of a known peptide synthetase and a partial amino acid sequence of the cyclic peptide synthetase purified from the fungus producing the substance PF1022, an appropriate primer is synthesized, which is used to effect polymerase chain reaction (PCR) with the genomic DNA derived from the fungus producing the substance PF1022 as template, to amplify a DNA fragment of the cyclic peptide synthetase gene. Using the DNA fragment as probe, the genome library is screened. In such manner, the whole region of the cyclic peptide synthetase gene can be isolated. After the determination of the nucleotide sequence of the DNA fragment, appropriate restriction cleavage sites are introduced upstream the translation initiation codon and downstream the translation termination codon by means such as PCR, to recover a gene fragment singly containing the cyclic peptide synthetase gene.

[0012]

The gene of the invention encompasses a nucleotide

sequence hybridizable with the thus determined nucleotide sequence under stringent conditions. Using routine methods (for example site-directed mutagenesis) in the field of genetic engineering, additionally, DNA fragments with modification of the gene, such as addition, insertion, deletion or substitution of the gene, can be encompassed within the scope of the invention. The stringent conditions herein referred to mean that the rinsing procedure of the membrane after hybridization is carried out in solutions at low salt concentrations and high temperature, for example, a condition such that rinsing is effected in 0.2 x SSC (1 x SSC: 15 mM citrate trisodium, 150 mM sodium chloride) - 0 .1 % SDS solution at 60°C for 15 minutes.

[0013]

A promoter is conjugated upstream the cyclic peptide synthetase gene isolated by the method, while a terminator is conjugated downstream the cyclic peptide synthetase gene; additionally, selective marker genes such as nutrient auxotrophic complementary genes or/and genes with chemical resistance are conjugated thereto, to prepare a recombinant vector for gene expression.

[0014]

The selective markers for use in recombinant vectors for gene expression include for example nutrient auxotrophic complementary genes such as pyrG, argB, trpC, niaD, TRP1, LEU2 and URA3; and genes with chemical resistance against destomycin, benomil, oligomycin, hygromycin, G418, bleomycin, fleomycin, phosphinothricin, ampicillin, and kanamycin.

[0015]

The conjugation of the promoter and terminator to the inventive gene and the insertion thereof into a vector can be carried out by methods known per se. The promoter and terminator for use in accordance with the invention are not

specifically limited, and include for example, genes of glycolytic enzymes, such as 3-phosphoglycerate kinase, glycelaldehyde-3-phosphate dehydrogenase and enolase; genes of amino acid synthesis, such as ornithine carbamoyltransferase and tryptophan synthase; genes of hydrolases, such as amylase, protease, lipase, cellulase and acetoamidase; genes of oxidoreductases, such as nitrate reductase, orotidine-5'-phosphate dehydrogenase, and alcohol dehydrogenase; and genes of bacteria producing the substance PF1022, which are highly expressed in the fungus producing the substance PF1022, such as Abp1.

[0016]

The transformation of a host with a recombinant vector prepared in such manner and the culturing of the resulting transformant enable prominent production of the substance PF1022. For a host with no synthesis of L-leucine, D-lactic acid or D-phenyllactic acid as a substrate for the cyclic depsipeptide synthetase of the invention, additionally, the host is cultured after addition of deficient substrates or derivatives thereof, whereby the substance PF1022 or derivatives thereof can be produced. The invention also encompasses the culturing of the transformant in a culture medium to collect the substance PF1022 or a derivative thereof from the resulting microorganisms.

[0017]

As the host for use, appropriate bacteria or fungal microorganisms usable as hosts for gene recombination can be used, with no specific limitation. Preferably, the host is Escherichia coli, a bacterium of the genus Bacillus, an actinomycetes, yeast and a filamentous fungus; more preferably, the host is a filamentous fungus producing the substance PF1022; and most preferably, the host is the strain PF1022 (Mycelia

sterilia, FERM BP-2671).

[0018]

The transformation of such host can be carried out by methods known per se. For example, the introduction of a recombinant vector for gene expression into a host can be carried out by routine methods, for example electroporation process, polyethylene glycol process, Agrobacterium process, lithium process, calcium chloride process and the like, with no specific limitation.

[0019]

The transformant can also be cultured by general methods, by appropriately selecting culture media and culturing conditions and the like. As the culture media, use can be made of routine components, such as carbon sources for example glucose, sucrose, thick malt syrup, dextrin, starch, glycerol, molasses, animal and vegetable oils and the like. As the nitrogen source, additionally, use can be made of soy bean powder, wheat germ, pharmamedia, corn steep liquor, cotton seed bran, meat extract, polypeptone, malt extract, yeast extract, ammonium sulfate, sodium nitrate, urea and the like. Additionally, it is effective to add inorganic salts capable of generating sodium, potassium, calcium (calcium carbonate and the like), magnesium, cobalt, chloride, phosphorus (dipotassium hydrogen phosphate), sulfuric acid (magnesium sulfate and the like) and other ions, if necessary. If necessary, furthermore, selective chemical agents including various vitamins such as thiamine (thiamine chloride salt and the like), amino acids such as glutamic acid (sodium glutamate and the like) and asparagine (DL-asparagine and the like), trace nutrients such as nucleotide and antibiotics can be added. Organic materials and inorganic materials supporting fungal growth and promoting the production of the substance PF1022 or

a derivative thereof can appropriately be added.

[0020]

The pH of the culture medium is about pH 6 to pH 8. As the culturing method, agitation culture, aerated agitation culture or submerged aerobic culture under aerobic conditions can be carried out. Particularly, submerged aerated culture is the most appropriate. The temperature appropriate for culturing is 15°C to 40°C. In many cases, the microorganism grows around 26°C to 37°C. The production of the substance PF1022 or a derivative thereof varies, depending on the culture medium and culture conditions or the host used, but the accumulation thereof generally reaches the peak in 2 days to 25 days by any of the culture methods. Just when the amount of the substance PF1022 or a derivative thereof reaches the peak, the culturing is terminated, to isolate and purify the objective substance from the culture.

[0021]

So as to recover the substance PF1022 or a derivative thereof from the culture, general separation means utilizing the characteristic properties, for example solvent extraction method, ion exchange resin method, adsorption or partition column chromatography method, gel filtration method, dialysis method, precipitation method and crystallization method, can be used singly or in appropriate combinations thereof to extract and purify the substance PF1022 or a derivative thereof. From the culture, for example, the substance PF1022 or a derivative thereof is extracted in acetone, methanol, butanol, ethyl acetate, butyl acetate and the like. So as to further purify the substance PF1022 or a derivative thereof, chromatography using Sephadex LH-20 (Pharmacia Co.) or Toyopearl HW-40 (Toso, Co.) is satisfactorily effected. By the methods described above or combinations thereof, the substance PF1022 or a

derivative thereof can be recovered in purity.

[0022]

[Examples]

The present invention will now be described in the following examples, but the invention is not limited to them.

[0023]

Example 1

Cloning of cyclic depsipeptide synthetase gene from a fungus producing the substance PF1022

1. Genomic DNA isolation and preparation of genome library

With UV irradiation or NTG treatment, mutation was induced into the fungus (Mycelia sterilia; FERM BP-2671) producing the substance PF1022, to prepare a fungal strain 432-26 producing the substance PF1022 and having an improved productivity of PF1022, from which the genome DNA was extracted. The fungal strain 432-26 producing the substance PF1022 was cultured in 50-ml of a seed culture medium [1 % yeast extract, 1 % malt extract, 2 % polypeptone, 2.5 % glucose, 0.1 % dipotassium hydrogen phosphate, 0.05 % magnesium sulfate (pH 7.0)] at 26°C for 2 days, to recover the fungi by centrifugation. The resulting fungi were frozen in liquid nitrogen and ground in a mortar with a pestle. From the ground fungi, the genome DNA was isolated by ISOPLANT (Nippon Gene, Co.) according to the attached protocol. The isolated genome DNA was partially digested with Sau3A I, to recover DNA fragments of 15 kb to 20 kb by agarose gel electrophoresis, which were then treated with alkali phosphatase to dephosphorylate the termini of the DNA fragments. The DNA fragments were inserted in a phage vector Lambda DASH II (STRATAGENE, CO.). The recombinant phage vector recovered in such manner was subjected to in vitro packaging with Gigapack III Gold Packaging Extract (STRATAGENE CO.) according to the attached protocol. Subsequently, the

recombinant phage grew on the host Escherichia coli strain XL1-Blue MRA (P2) for plaque formation on a plate.

[0024]

2. Isolation of partial DNA fragment of cyclic depsipeptide synthetase gene

A known peptide synthetase was subjected to multiple alignment, so that WTSMYDG and VVQYFPT were detected as excellently conserved regions. Based on these sequences, primers 5'-TGGACIWSNATGTAYGAYGG-3' (SQ ID NO. 3) and 5'-GTIGGRAARTAYTGIACNAC-3'(SQ ID NO. 4) were synthesized. Using these primers, the genome DNA isolated from the fungus producing the substance PF1022 was used as template for PCR. Using 50 ng of the genome DNA as template, 1 .25 units ExTaq DNA polymerase (TaKaRa Brewery, Co.), the attached buffer and dNTP mixture, and 10 μM primer in 50 μl of a reaction solution, PCR was conducted under the following conditions: 94°C for 3 minutes [94°C for one minute, 65°C (with 0.5°C decrement per one cycle) for one minute, 72°C for one minute] x 30 times and 72°C for 3 minutes. Through the reaction, a DNA fragment of about 350 bp was amplified; and the resulting DNA fragment was inserted in pCR2.1 plasmid vector, by using Original TA Cloning Kit (Invitrogen Co.) according to the attached protocol.

[0025]

The nucleotide sequence of the thus cloned DNA fragment was determined, by using DNA sequencing Kit dRhodamine Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Co.) and ABI PRISM 310 Genetic Analyzer (Perkin Elmer Co.) according to the attached protocol. Consequently, the nucleotide sequence of the isolated DNA fragment was homologous to the peptide synthetase gene, which apparently indicates that the DNA fragment was a part of the objective cyclic depsipeptide synthetase gene.

[0026]

3. Cloning of the whole region of the cyclic depsipeptide synthetase gene

The probe for use in the screening of the genome library was prepared, by PCR to allow the DNA fragment to incorporate fluorescein-labeled dUTP. By using pCR2.1 plasmid vector inserted with 100 ng of the DNA fragment of the cyclic depsipeptide synthetase gene as template, 1.25 units ExTaq DNA polymerase (TaKaRa Brewery, Co.) and the attached buffer, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.02 mM dTTP, 0.18 mM fluorescein-labeled dUTP (FluoroGreen; Amersham Co.) and 10μM primers (SQ ID Nos. 3 and 4) in 50 μl of a reaction solution, PCR was carried out under the following conditions: 94°C for 2 minutes, (94°C for 30 seconds, 55°C for one minute and 72°C for one minute) x 25 times, 72°C for 3 minutes. Through the reaction, a fluorescein-labeled probe of about 350 bp was prepared.

[0027]

On the plate with formed plaques as prepared in Example 1.1, Hybond-N+ membrane (Amersham Co.) was mounted, to deposit the plaques. The membrane was treated with an alkali, to denature the recombinant phage DNA on the membrane into a single strand and thereby adsorb the DNA on the membrane. The phage DNA-adsorbed membrane was placed in a buffer prepared by using Hybridization Buffer Tablets (Amersham Co.), for incubation at 60°C for one hour. The fluorescein-labeled probe was denatured and added to the resulting incubation mixture, for overnight hybridization at 60°C. Thereafter, the membrane was rinsed in 1 x SSC (SSC: 15 mM citrate trisodium, 150 mM sodium chloride) -0.1 % SDS solution at 60°C for 15 minutes and further rinsed in 0.2 x SSC-0.1 % SDS solution at 60°C for 15 minutes. The

fluorescein-bound plaque was visualized by using DIG-wash and block buffer set (Boehringer Mannheim, Co.), alkali phosphatase-labeled anti-fluorescein antibody (Anti-fluorescein-AP, Fab fragment, Boehringer Mannheim Co.), a chromogenic substrate nitroblue tetrazolium chloride (Boehringer Mannheim Co.) and X-phosphate (Boehringer Mannheim Co.) according to the attached protocol. In such manner, a positive clone carrying the 5' upstream region and 3' downstream region of a region homologous to the probe was screened.

[0028]

4. Determination of the nucleotide sequence

The DNA fragment in the positive clone thus isolated was amplified by PCR using primers 5'-GCGGAATTAACCCTCACTAAAGGGAACGAA-3' (SQ ID No. 5) and 5'-GCGTAATACGACTCACTATAGGGCGAAGAA-3' (SQ ID No. 6) as the phage vector sequences. Using 100 ng of the positive clone DNA as template, 2.5 units LA Taq DNA polymerase (TaKaRa Brewery, Co.), the attached buffer and dNTP mixture, 2.5 mM magnesium chloride, and 0.2 μM primer in a reaction solution of 50μl, PCR was effected under the following conditions: 94°C for one minute, (98°C for 10 seconds and 68°C for 15 minutes) x 25 times, 72°C for 15 minutes. The resulting PCR product was purified and treated with a nebulizer, to be decomposed randomly into fragments of 0.5 kb to 2.0 kb. The termini of the resulting fragments were blunt ended with T4 DNA polymerase and phosphorylated with T4 polynucleotide kinase, to be then inserted at the EcoRV site of pT7Blue (Novagen Co.) for insertion in Escherichia coli strain JM109. 168 colonies thus prepared were directly subjected to PCR using M13 Primer M4 (TaKaRa Brewery, Co.) and M13 primer RV (TaKaRa Brewery, Co.) and were then purified, which were then sequenced by using M13 primer M4 (TaKaRa Brewery, Co.). Using 1.25 units ExTaq DNA polymerase (TaKaRa Brewery,

Co.), the attached buffer and dNTP mixture, and 0.5 μM primer, PCR was effected in 50 μl of a reaction solution under the following conditions: 94°C for 4 minutes, (94°C for 30 seconds, 55°C for 30 seconds and 72°C for 2 minutes) x 30 times, 72°C for 3 minutes. Additionally, sequencing was effected, by using DNA Sequencing Kit dRhodamine Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Co.) and ABI PRISM 310 Genetic Analyzer (Perkin Elmer Co.) according to the attached protocol.

[0029]

Based on the results, an insufficiently analyzed region was amplified by PCR using a primer newly designed on the basis of the analyzed nucleotide sequence and was then purified. Using the primer used for PCR, the region was sequenced, whereby the 15606-bp nucleotide sequence of the DNA fragment in the positive clone was determined.

[0030]

The analysis of the sequence apparently indicates that an open reading frame (ORF) of 9633 bp was present and a protein speculated from the sequence comprised 3210 amino acid residues of 353 kDa and that the protein was homologous to the peptide synthetase. Additionally, the protein with the highest homology was enniatin synthetase (S39842) with 56 % homology. The nucleotide sequence and amino acid sequence of the ORF of the cyclic depsipeptide synthetase gene thus isolated in accordance with the invention are shown as SQ ID Nos. 1 and 2, respectively in the sequence listing.

[0031]

Example 2

Improvement of PF1022 productivity due to the excess expression of cyclic depsipeptide synthetase gene

1. Construction of recombinant vector for gene expression (Figure 1)

From the positive clone recovered in Example 1. 3, plasmid pPF7 was prepared by cleaving the inserted DNA fragment with NotI and inserting the DNA fragment into the NotI site of the pBluescriptII KS+ (STRATAGENE CO.). pPF7 was cleaved with BanIII and SmaI and elecrophoresed on agarose gel, to recover a DNA fragment of about 8250 by from the agarose gel. The fragment was inserted in pBluescriptII KS+, to prepare plasmid pPF7-1.

[0032]

Using pPF7 as template, 5'-AGCATCGGATCCTAACAATGGGCGTTGAGCAGCAAGCCCTA-3' (SQ ID No. 7; designed for the initiation of the translation at the 9th Met from the N terminus of the ORF) or 5'-AGCATCGGATCCTAACAATGTCAAACATGGCACCACTCCCTA-3' (SQ ID No. 13; designed for the initiation of the translation at the first Met from the N terminus of the ORF), and 5'-TTTGCTTCGTACTCGGGTCCT-3'(SQ ID No. 8) as primers for the amplification of a region of about 440 bp (using SQ ID Nos. 7 and 8) or a region of about 470 bp (using SQ ID Nos. 13 and 8) around the N terminus to the BanIII site, and 5'-GCATCGCGATACTAGAGAAG-3' (SQ ID No. 9) and 5'-AGCATCGAATTCGGATCCCTAAACCAACGCCAAAGCCCGAAT-3' (SQ ID No. 10) as primers for the amplification of a region of about 920 bp from the SmaI site to the C terminus, PCR was effected. Then, the primers were designed so as to insert the BamHI site at the 5' and 3' sites of the inventive cyclic depsipeptide synthetase gene. Using 150 ng of the plasmid DNA as template, 2.5 units KOD DNA polymerase (Toyo Boseki), the attached buffer and dNTP mixture, 1 mM magnesium chloride, and 0.5 μM primer in 50 μl of a reaction solution, PCR was effected under the following conditions : 98°C for 30 seconds, (98°C for 15 seconds, 65°C for 2 seconds, 74°C for 30 seconds) x 10 times, 74°C for one minute.

The PCR reaction solutions recovered by using the individual primers were precipitated with ethanol, to recover PCR products. Concerning the N terminal region, the N terminal region was cleaved with BamHI and BanIII; concerning the C terminal region, the C terminal region was cleaved with SmaI and BamHI. Thereafter, the resulting fragments were electrophoresed on agarose gel, to recover DNA fragments from the agarose gel.
[0033]

The PCR fragment of the C terminal region was inserted in the SmaI and BamHI sites of pPF7-1, to prepare plasmid pPF7-2. The plasmid was cleaved with BanIII and BamHI and electrophoresed on agarose gel, to recover a DNA fragment of about 9170 bp from the agarose gel. The DNA fragment and the N terminal region prepared by using SQ ID Nos. 7 and 8 were simultaneously inserted in the BamHI site of pBluescript II KS+, to reconstruct the cyclic depsipeptide synthetase gene of the invention and prepare plasmid pPFsyn (initiating the translation at the 9th Met from the N terminus of the ORF).
[0034]

Alternatively, an about 9170-bp DNA fragment cleaved from pPF7-2 and the N-terminal region prepared by using SQ ID Nos. 7 and 13 were simultaneously inserted in the BamHI site of pHSG299 (TaKaRa Brewery Co.), to reconstruct the cyclic depsipeptide synthetase gene of the invention and prepare plasmid pPFsynN (initiating the translation at the first Met from the N terminus of the ORF). In such manner, the cyclic depsipeptide synthetase gene with the BamHI sites on both the termini was prepared.
[0035]

Herein, Escherichia coli transformed with the plasmid pPFsyn (Escherichia coli DH5α) was deposited with Accession No. FERM P-17541 at National Institute of Bioscience and

Human-Technology, Agency of Industrial Science and Technology, supra.

[0036]

Additionally, Escherichia coli transformed with the plasmid pPFsynN (Escherichia coli DH5α) was deposited with Accession No. FERM P-17542 at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, supra.

[0037]

pPFsyn or pPFsynN was cleaved with BamHI. Subsequently, the cyclic depsipeptide synthetase gene region was recovered from the individual gels. The gene region was inserted at the BamHI site of pABPd with the promoter and terminator of Abp1 gene described in Japanese Patent Application No. 252851/1999, to prepare an expression vector pABP/PFsyn (initiating the translation at the 9th Met of the N terminus of ORF) and an expression vector pABP/PFsynN (initiating the translation at the first Met of the N terminus of ORF) as the expression vectors for expressing the cyclic depsipeptide synthetase gene of the invention.

[0038]

2. Introduction and expression of cyclic depsipeptide synthetase gene in fungus producing the substance PF1022

The expression vectors were introduced in a fungus producing the substance PF1022 (Mycelia sterilia; FERM BP-2671) according to the method of Example 1 described in WO 97/00944, to screen strains with high resistance against hygromycin B. The introduction of the objective gene in these strains was confirmed by PCR using a primer 5'-TGATATGCTGGAGCTTCCCT-3' (SQ ID No. 11) prepared from the sequence of Abp1 promoter and a primer 5'-GCACAACCTCTTTCCAGGCT-3' (SQ ID No. 12) prepared from the sequence of the cyclic depsipeptide synthetase gene. In

such manner, gene-introduced strains with high resistance against hygromycin B and with the inventive cyclic depsipeptide synthetase gene introduced therein were screened.

[0039]

The gene-introduced strains and the parent strain (Mycelia sterilia; FERM BP-2671) were separately cultured in 50 ml of a seed culture medium at 26°C for 2 days; 1 ml of each of the cultures was inoculated separately in a generation culture medium [6 % thick malt syrup, 2.6 % starch, 2 % wheat germ, 1 % pharmamedia, 0.2 % magnesium sulfate 7 hydrates, 0.2 % calcium carbonate, 0.3 % sodium chloride (pH 7 .5)] and cultured therein at 26°C for 4 days. The resulting culture was centrifuged at 4500 rpm for 5 minutes to harvest the fungus; the resulting individual fungus species were rinsed in 0.3 M potassium chloride. The individual fungal species were frozen in liquid nitrogen and freeze-dried.

[0040]

The following extraction procedure was carried out on ice or in a low-temperature chamber at 4°C. Into a 2-ml tube containing 10 mg of the freeze-dried fungal species and 1.0 ml of glass beads (0.5-mm diameter) was added 1.0 ml of an extraction buffer [50 mM Tris-HC1 (pH 8.0), 0.3 M potassium chloride, 60 % glycerol, 10 mM ethylenediaminetetraacetate disodium, 5 mM dithiothreitol, 10 µM leupeptin, 1 mM phenylmethanesulfonic acid, 60 µg/ml chimostatin]. The microtube was set in a Mini-Bead-Beater-8 (Biospec, Co.), which was then driven at the maximum speed for 3 minutes for extraction. The resulting mixture was centrifuged at 15000 rpm for 5 minutes; 100 µl of the supernatant was charged in and mixed with 100 µl of 10 % trichloroacetic acid solution. After the solution was left to stand for 15 minutes, the solution was centrifuged at 15000 rpm for 10 minutes. The resulting

precipitate was dissolved in 15 µl of an alkali solution (2 % sodium carbonate, 0.4 % sodium hydroxide), to which was added 60 µl of a sample buffer [125 mM Tris-HC1 (pH 6 . 8), 20 % glycerol, 4 % sodium dodecylsulfate, 10 % 2-mercaptoethanol, 50 mg/1 bromophenol blue]. This was heated in boiling water for 5 minutes, and was then electrophoresed [Sodium Dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)] on 4 % to 20 % polyacrylamide gel with an electrophoresis system (Tefco Co.). The polyacrylamide gel after electrophoresis was stained by using Quick-CBB (Wako Pure Chemical Co.) according to the attached protocol. The electrophoresis result of the proteins extracted from the parent strain and the gene-introduced strain with pABP/PFsyn is shown in Figure 2.

[0041]

As described above, the expression level of the cyclic depsipeptide synthetase in the gene-introduced strains was distinctively higher than the level in the parent strain.

[0042]

3. Extraction and assay of the substance PF1022

The gene-introduced strains and the parent strain were separately cultured in 50 ml of a seed culture medium at 26°C for 2 days; and 1 ml of each of 50 ml of the cultures was inoculated separately in a generation culture medium and cultured therein at 26°C for 6 days. 10 ml each of the cultures was placed and centrifuged at 3000 rpm for 10 minutes; and the resulting strains were harvested separately. 10 ml of methanol was added to the individual strains, which were vigorously shaken and left to stand for 30 minutes. Thereafter, those were again shaken and centrifuged at 3000 rpm for 10 minutes; the substance PF1022 extracted from the individual strains in the supernatant was assayed by liquid chromatography. As the column, LiChrospher 100 RP-18 (e) (Kanto Chemical C0.) was used;

the column temperature was 40°C; the mobile phase was 80 % acetonitrile at a flow rate of 1.0 ml/min; the substance PF1022 was detected on the basis of the absorbance at 210 nm. The retention time of the substance PF1022 under the conditions was 5 minutes to 6 minutes. The experiments were carried out in duplicate. The average values of the assay results of the substance PF1022 extracted from the parent strain and the gene-introduced strain with the pABP/PFsyn are shown in Table 1.

[0043]

[Table 1]

	Substance PF1022 (μg/ml)
Parent strain	88
Gene-introduced strain	222

[0044]

The substance PF1022 productivity of the gene-introduced strain was about 2.5-fold that of the parent strain. It is apparently shown that the excess expression of the inventive cyclic depsipeptide synthetase elevates the productivity of the substance PF1022.

[0045]

[ADVANTAGEOUS EFFECTS OF THE INVENTION]

The introduction of the inventive cyclic depsipeptide synthetase gene in the fungus producing the substance PF1022 can improve the productivity of the substance PF1022.

[0046]

[SEQUENCE LISTING]

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caa ctc aac ggc cat aga gag cgt aac aat tat tta gat cta aat ggg 5232

Gln Leu Asn Gly His Arg Glu Arg Asn Asn Tyr Leu Asp Leu Asn Gly

1725 1730 1735 1740

cac tca ctg cta aat ggg aat tca gac atc acc aca tca ggg ccc tca 5280

His Ser Leu Leu Asn Gly Asn Ser Asp Ile Thr Thr Ser Gly Pro Ser

1745 1750 1755

gca aca agc ctt gcc tac gtg atc ttc aca tcc ggc tca acc gga aag 5328

Ala Thr Ser Leu Ala Tyr Val Ile Phe Thr Ser Gly Ser Thr Gly Lys

1760 1765 1770

ccc aaa gga gtc atg gtc gaa cac cgc agc atc atc cga ctt gca aag 5376

Pro Lys Gly Val Met Val Glu His Arg Ser Ile Ile Arg Leu Ala Lys

1775 1780 1785

aag aac aga atc ata tcc agg ttc cca tct gta gcc aag gta gct cac 5424

Lys Asn Arg Ile Ile Ser Arg Phe Pro Ser Val Ala Lys Val Ala His

1790 1795 1800

ctc tca aac atc gcc ttt gac gcc gcc act tgg gaa atg ttc gca gcc 5472

Leu Ser Asn Ile Ala Phe Asp Ala Ala Thr Trp Glu Met Phe Ala Ala

1805 1810 1815 1820

ctt cta aac ggc gga acg ctg gtc tgt atc gac tat atg acc acc ctg 5520

Leu Leu Asn Gly Gly Thr Leu Val Cys Ile Asp Tyr Met Thr Thr Leu

1825 1830 1835

gac agc aaa acg ctc gag gcc gcg ttt gca cga gaa caa atc aac gcc 5568

Asp Ser Lys Thr Leu Glu Ala Ala Phe Ala Arg Glu Gln Ile Asn Ala

1840 1845 1850

gcg tta ctc acg ccc gct ttg ttg aag cag tgc cta gcc aac att ccc 5616

Ala Leu Leu Thr Pro Ala Leu Leu Lys Gln Cys Leu Ala Asn Ile Pro

1855 1860 1865

act acc cta ggc agg ctg agt gca ctc gtt att gga ggt gat agg ctt 5664

Thr Thr Leu Gly Arg Leu Ser Ala Leu Val Ile Gly Gly Asp Arg Leu

1870 1875 1880

gac ggc caa gac gcg atc gca gca cat gcg ctt gtc ggt gct ggc gtg 5712

Asp Gly Gln Asp Ala Ile Ala Ala His Ala Leu Val Gly Ala Gly Val

1885 1890 1895 1900

tat aat gcg tat ggc ccg acc gaa aac gga gtg atc agt acg att tat 5760

Tyr Asn Ala Tyr Gly Pro Thr Glu Asn Gly Val Ile Ser Thr Ile Tyr

1905 1910 1915

aat atc act aaa aac gac tcg ttc atc aac gga gtc ccc atc ggc tgt 5808

Asn Ile Thr Lys Asn Asp Ser Phe Ile Asn Gly Val Pro Ile Gly Cys

1920 1925 1930

gca atc agc aat tcc ggc gcc tac atc aca gac cca gac cag cag ctc 5856

Ala Ile Ser Asn Ser Gly Ala Tyr Ile Thr Asp Pro Asp Gln Gln Leu

1935 1940 1945

gta cct cct ggc gtc atg ggt gaa ctc gtc gtt acc ggt gac ggg ctc 5904

Val Pro Pro Gly Val Met Gly Glu Leu Val Val Thr Gly Asp Gly Leu

1950 1955 1960

gcg cgg ggg tat aca gac cca gca cta gac gcg ggc cgc ttc gtc cag 5952

Ala Arg Gly Tyr Thr Asp Pro Ala Leu Asp Ala Gly Arg Phe Val Gln

1965 1970 1975 1980

atc atg atc aat gac aag gcc gtg agg gcg tac cga acg ggt gac cgg 6000

Ile Met Ile Asn Asp Lys Ala Val Arg Ala Tyr Arg Thr Gly Asp Arg

1985 1990 1995

gca cga tat cgc gta gga gac ggt cag atc gag ttc ttc gga cgc atg 6048

Ala Arg Tyr Arg Val Gly Asp Gly Gln Ile Glu Phe Phe Gly Arg Met

2000 2005 2010

gat cag caa gtc aag atc cga ggt cac cgc att gaa cca gcc gaa gtg 6096

Asp Gln Gln Val Lys Ile Arg Gly His Arg Ile Glu Pro Ala Glu Val

2015 2020 2025

gag cgt gct att ctc gac caa gac tcg gcc cgc gac gcc gtc gtt gtc 6144

Glu Arg Ala Ile Leu Asp Gln Asp Ser Ala Arg Asp Ala Val Val Val

2030 2035 2040

atc cgg cac caa gaa ggt gaa gaa ccg gag atg gtt ggt ttc gtc gcg 6192

Ile Arg His Gln Glu Gly Glu Glu Pro Glu Met Val Gly Phe Val Ala

2045 2050 2055 2060

acc cac ggc gat cac tct gcc gaa caa gag gaa gca gac gac cag gtt 6240

Thr His Gly Asp His Ser Ala Glu Gln Glu Glu Ala Asp Asp Gln Val

2065 2070 2075

gaa ggt tgg aaa gac ttc ttc gag agc aat aca tat gcc gac atg gat 6288

Glu Gly Trp Lys Asp Phe Phe Glu Ser Asn Thr Tyr Ala Asp Met Asp

2080 2085 2090

acc atc ggc cag tct gct ata ggc aac gac ttt acg ggc tgg acg tcc 6336

Thr Ile Gly Gln Ser Ala Ile Gly Asn Asp Phe Thr Gly Trp Thr Ser

2095 2100 2105

atg tac gac ggg agc gag atc aac aag gcc gag atg cag gag tgg ctc 6384

Met Tyr Asp Gly Ser Glu Ile Asn Lys Ala Glu Met Gln Glu Trp Leu

2110 2115 2120

gac gac acc atg cgc aca ctc ctc gat ggc caa gcg ccg ggt cac gta 6432

Asp Asp Thr Met Arg Thr Leu Leu Asp Gly Gln Ala Pro Gly His Val

2125 2130 2135 2140

ctc gaa ata ggc aca ggc agt ggc atg gta ttg ttt aac tta ggg gcc 6480

Leu Glu Ile Gly Thr Gly Ser Gly Met Val Leu Phe Asn Leu Gly Ala

2145 2150 2155

ggg cta caa agc tac gta ggt ctt gaa cca tct aga tct gca gcc acg 6528

Gly Leu Gln Ser Tyr Val Gly Leu Glu Pro Ser Arg Ser Ala Ala Thr

2160 2165 2170

ttt gtt acc aaa gcg atc aat tcc acc cca gct ctt gca gga aag gcc 6576

Phe Val Thr Lys Ala Ile Asn Ser Thr Pro Ala Leu Ala Gly Lys Ala

2175 2180 2185

gaa gtg cac gtc ggc aca gcg aca gac ata aac cga ctt cgt gga ctt 6624

Glu Val His Val Gly Thr Ala Thr Asp Ile Asn Arg Leu Arg Gly Leu

2190 2195 2200

cgt ccc gat cta gtt gtg ctc aac tcg gta gtc cag tat ttc ccc acg 6672

Arg Pro Asp Leu Val Val Leu Asn Ser Val Val Gln Tyr Phe Pro Thr

2205 2210 2215 2220

ccc gag tac cta cta gag gtc gtg gag agt ctc gtc cgg att ccg ggc 6720

Pro Glu Tyr Leu Leu Glu Val Val Glu Ser Leu Val Arg Ile Pro Gly

2225 2230 2235

gtc aag cgc gtg gtc ttc ggc gac ata cga tct cac gcc acg aac aga 6768

Val Lys Arg Val Val Phe Gly Asp Ile Arg Ser His Ala Thr Asn Arg

2240 2245 2250

cat ttt ctt gct gcc agg gcg ctg cat tcg ctg ggc tcc aag gcg acc 6816

His Phe Leu Ala Ala Arg Ala Leu His Ser Leu Gly Ser Lys Ala Thr

2255 2260 2265

aaa gat gct ata cgt caa aag atg acg gag atg gaa gag cgc gag gaa 6864

Lys Asp Ala Ile Arg Gln Lys Met Thr Glu Met Glu Glu Arg Glu Glu

2270 2275 2280

gag ctg ctc gtc gac ccg gcc ttc ttc acg gcg ctg ctg cag ggc cag 6912

Glu Leu Leu Val Asp Pro Ala Phe Phe Thr Ala Leu Leu Gln Gly Gln

2285 2290 2295 2300

ctt gcc gat cga atc aag cac gtc gag atc ctc ccg aag aac atg cgc 6960

Leu Ala Asp Arg Ile Lys His Val Glu Ile Leu Pro Lys Asn Met Arg

2305 2310 2315

gcc acg aac gag ctg agc gcg tac cgg tat aca gcc gtc att cac gta 7008

Ala Thr Asn Glu Leu Ser Ala Tyr Arg Tyr Thr Ala Val Ile His Val

2320 2325 2330

cgc ggc cca gag gaa cag tcg cgg ccc gtg tat ccg atc caa gtg aac 7056

Arg Gly Pro Glu Glu Gln Ser Arg Pro Val Tyr Pro Ile Gln Val Asn

2335 2340 2345

gac tgg atc gac ttt cag gcc tca cgc att gac cgc cgc gcc ctt ctc 7104

Asp Trp Ile Asp Phe Gln Ala Ser Arg Ile Asp Arg Arg Ala Leu Leu

2350 2355 2360

cga ctc cta cag cgc tcg gca gac gcc gcg acc gtc gcc gtc agc aac 7152

Arg Leu Leu Gln Arg Ser Ala Asp Ala Ala Thr Val Ala Val Ser Asn

2365 2370 2375 2380

atc ccc tac agc aag acg att gta gaa cgc cat gtc gtc gag tcc ctt 7200

Ile Pro Tyr Ser Lys Thr Ile Val Glu Arg His Val Val Glu Ser Leu

2385 2390 2395

gac aat aac aac agg gag aat acg cat aga gca cca gac ggc gcg gct 7248

Asp Asn Asn Asn Arg Glu Asn Thr His Arg Ala Pro Asp Gly Ala Ala

2400 2405 2410

tgg atc tcg gcc gtc cgc tcc aag gcc gag cgc tgc acg tcc ctc tcc 7296

Trp Ile Ser Ala Val Arg Ser Lys Ala Glu Arg Cys Thr Ser Leu Ser

2415 2420 2425

gtg acc gat ctt gtg cag ctc ggg gaa gaa gcc ggc ttt cgc gta gaa 7344

Val Thr Asp Leu Val Gln Leu Gly Glu Glu Ala Gly Phe Arg Val Glu

2430 2435 2440

gtc agc gca gcg cgg cag tgg tct caa agc ggc gcg ctc gat gcc gtc 7392

Val Ser Ala Ala Arg Gln Trp Ser Gln Ser Gly Ala Leu Asp Ala Val

2445 2450 2455 2460

ttt cac cgc tat aat ttg ccc act caa agc aat agt cgc gtt ctg att 7440

Phe His Arg Tyr Asn Leu Pro Thr Gln Ser Asn Ser Arg Val Leu Ile

2465 2470 2475

cag ttc cct acc gaa gat ggc cag acg cga aga tcc gcc act ctg aca 7488

Gln Phe Pro Thr Glu Asp Gly Gln Thr Arg Arg Ser Ala Thr Leu Thr

2480 2485 2490

aac cga cca cta cag cgt ctg cag agc cgt cgg ttc gca tca cag atc 7536

Asn Arg Pro Leu Gln Arg Leu Gln Ser Arg Arg Phe Ala Ser Gln Ile

2495 2500 2505

cgc gaa cag ctg aag gcc gtg ctc ccg tca tac atg atc ccc tcc cgc 7584

Arg Glu Gln Leu Lys Ala Val Leu Pro Ser Tyr Met Ile Pro Ser Arg

2510 2515 2520

atc gtg gtc ata gac cag atg cct ctc aat gcc aat ggc aag gtc gac 7632

Ile Val Val Ile Asp Gln Met Pro Leu Asn Ala Asn Gly Lys Val Asp

2525 2530 2535 2540

cgg aaa gaa ctt acc aga agg gcc caa atc gcg ccg aaa tct cag gcg 7680

Arg Lys Glu Leu Thr Arg Arg Ala Gln Ile Ala Pro Lys Ser Gln Ala

2545 2550 2555

gct ccc gcc aaa ccc gtc aaa cag gtc gat ccg ttc gtc aac ctg gaa 7728

Ala Pro Ala Lys Pro Val Lys Gln Val Asp Pro Phe Val Asn Leu Glu

2560 2565 2570

gcc att tta tgt gag gag ttc gcg gag gtg ctg ggc atg gaa gtc ggc 7776

Ala Ile Leu Cys Glu Glu Phe Ala Glu Val Leu Gly Met Glu Val Gly

2575 2580 2585

gtg aac gac cac ttc ttc caa cta ggc gga cac tct ctc ttg gcc acg 7824

Val Asn Asp His Phe Phe Gln Leu Gly Gly His Ser Leu Leu Ala Thr

2590 2595 2600

aag ctc gtc gcg cgt ctc agt cgt cgg cta aac ggt cgt gtg tct gtg 7872

Lys Leu Val Ala Arg Leu Ser Arg Arg Leu Asn Gly Arg Val Ser Val

2605 2610 2615 2620

agg gat gtg ttc gac cag cct gtg att tcc gac ctc gca gtc acc ctc 7920

Arg Asp Val Phe Asp Gln Pro Val Ile Ser Asp Leu Ala Val Thr Leu

2625 2630 2635

cgc cag gga ctg acc ttg gaa aac gcc att ccc gca acg ccg gac agc 7968

Arg Gln Gly Leu Thr Leu Glu Asn Ala Ile Pro Ala Thr Pro Asp Ser

2640 2645 2650

ggg tat tgg gag cag aca atg tcc gca ccg aca acc ccg agc gac gac 8016

Gly Tyr Trp Glu Gln Thr Met Ser Ala Pro Thr Thr Pro Ser Asp Asp

2655 2660 2665

atg gag gcc gtg cta tgc aag gag ttt gcg gat gtg ctt ggc gtc gaa 8064

Met Glu Ala Val Leu Cys Lys Glu Phe Ala Asp Val Leu Gly Val Glu

2670 2675 2680

gtc agc gcc acc gac agc ttc ttc gat ctc ggt ggg cat tcc ctc atg 8112

Val Ser Ala Thr Asp Ser Phe Phe Asp Leu Gly Gly His Ser Leu Met

2685 2690 2695 2700

gct acg aag ctc gct gcg cgt att agc cgt cgg cta gat gta ccg gtg 8160

Ala Thr Lys Leu Ala Ala Arg Ile Ser Arg Arg Leu Asp Val Pro Val

2705 2710 2715

tca atc aaa gac ata ttc gat cac tca gtt cct cta aac ctt gcg agg 8208

Ser Ile Lys Asp Ile Phe Asp His Ser Val Pro Leu Asn Leu Ala Arg

2720 2725 2730

aag att cgg ctc act caa gca aaa ggc cac gaa gcg acc aat gga gta 8256

Lys Ile Arg Leu Thr Gln Ala Lys Gly His Glu Ala Thr Asn Gly Val

2735 2740 2745

caa atc gcc aac gac gcc cca ttc caa ctc att tcc gta gaa gat cca 8304

Gln Ile Ala Asn Asp Ala Pro Phe Gln Leu Ile Ser Val Glu Asp Pro

2750 2755 2760

gag ata ttc gtc caa cgt gaa atc gcc cct caa cta caa tgc tca ccc 8352

Glu Ile Phe Val Gln Arg Glu Ile Ala Pro Gln Leu Gln Cys Ser Pro

2765 2770 2775 2780

gag aca atc cta gac gtc tac ccc gcc acg caa atg caa agg gtc ttc 8400

Glu Thr Ile Leu Asp Val Tyr Pro Ala Thr Gln Met Gln Arg Val Phe

2785 2790 2795

ctc ctc aac cca gta aca gga aag ccg cgc tca cca acg cca ttt cac 8448

Leu Leu Asn Pro Val Thr Gly Lys Pro Arg Ser Pro Thr Pro Phe His

2800 2805 2810

ata gac ttc ccg ccg gac gca gac tgc gcc agc ctg atg cgg gca tgt 8496

Ile Asp Phe Pro Pro Asp Ala Asp Cys Ala Ser Leu Met Arg Ala Cys

2815 2820 2825

gca tct cta gcg aag cat ttc gat atc ttc agg acg gtg ttc ctc gaa 8544

Ala Ser Leu Ala Lys His Phe Asp Ile Phe Arg Thr Val Phe Leu Glu

2830 2835 2840

gcc aga ggc gaa ctc tac caa gta gtt cta aaa cac gtc gac gtg ccc 8592

Ala Arg Gly Glu Leu Tyr Gln Val Val Leu Lys His Val Asp Val Pro

2845 2850 2855 2860

atc gag atg ctc cag acc gaa gaa aac atc aac agc gcg acc cgg tcg 8640

Ile Glu Met Leu Gln Thr Glu Glu Asn Ile Asn Ser Ala Thr Arg Ser

2865 2870 2875

ttc ctg gac gta gac gca gaa aaa ccc atc cgg cta ggc cag cca ctg 8688

Phe Leu Asp Val Asp Ala Glu Lys Pro Ile Arg Leu Gly Gln Pro Leu

2880 2885 2890

atc cgc atc gcg ata cta gag aag ccc ggg tcc acg ctg cgc gtc atc 8736

Ile Arg Ile Ala Ile Leu Glu Lys Pro Gly Ser Thr Leu Arg Val Ile

2895 2900 2905

cta cga tta tcc cac gcc tta tac gac ggc ctg agc cta gag cac atc 8784

Leu Arg Leu Ser His Ala Leu Tyr Asp Gly Leu Ser Leu Glu His Ile

2910 2915 2920

ctg cac tct ctg cac atc ctc ttt ttc ggc ggc agt ctt ccc ccg ccg 8832

Leu His Ser Leu His Ile Leu Phe Phe Gly Gly Ser Leu Pro Pro Pro

2925 2930 2935 2940

ccc aag ttc gcc ggg tac atg caa cac gtc gcg agc agt cgc aga gaa 8880

Pro Lys Phe Ala Gly Tyr Met Gln His Val Ala Ser Ser Arg Arg Glu

2945 2950 2955

ggc tac gat ttc tgg cgt tcc gtt ctc cga gat tcg tct atg aca gtc 8928

Gly Tyr Asp Phe Trp Arg Ser Val Leu Arg Asp Ser Ser Met Thr Val

2960 2965 2970

atc aaa ggc aac aat aat aca act cca cca cct cct cct caa caa caa 8976

Ile Lys Gly Asn Asn Asn Thr Thr Pro Pro Pro Pro Pro Gln Gln Gln

2975 2980 2985

tcc acc ccc tcc gga gcc cac cac gcc tcc aaa gta gtc act atc cca 9024

Ser Thr Pro Ser Gly Ala His His Ala Ser Lys Val Val Thr Ile Pro

2990 2995 3000

acc caa gcc aac aca gac agc cgg atc acg cgc gcc acg atc ttc act 9072

Thr Gln Ala Asn Thr Asp Ser Arg Ile Thr Arg Ala Thr Ile Phe Thr

3005 3010 3015 3020

acc gct tgc gca cta atg ctc gcg aaa gaa gac aac tcc agc gac gtc 9120

Thr Ala Cys Ala Leu Met Leu Ala Lys Glu Asp Asn Ser Ser Asp Val

3025 3030 3035

gtc ttc ggg cgt acg gta tcg ggg cgt caa ggc ctg ccc cta gcc cac 9168

Val Phe Gly Arg Thr Val Ser Gly Arg Gln Gly Leu Pro Leu Ala His

3040 3045 3050

caa aac gtg atc gga cca tgt ctc aac caa gtg ccc gtg cgc gcg cgc 9216

Gln Asn Val Ile Gly Pro Cys Leu Asn Gln Val Pro Val Arg Ala Arg

3055 3060 3065

ggt tta aac cga gga acc act cac cac cga gaa ctt ctc cgc gag atg 9264

Gly Leu Asn Arg Gly Thr Thr His His Arg Glu Leu Leu Arg Glu Met

3070 3075 3080

caa gag caa tat ctc aac agt ctc gct ttc gaa act ctc ggg tac gac 9312

Gln Glu Gln Tyr Leu Asn Ser Leu Ala Phe Glu Thr Leu Gly Tyr Asp

3085 3090 3095 3100

gag atc aag gcg cac tgc aca gac tgg ccg gac gtg cca gcg acc gcg 9360

Glu Ile Lys Ala His Cys Thr Asp Trp Pro Asp Val Pro Ala Thr Ala

3105 3110 3115

agc ttc ggg tgc tgc atc gtg tac cag aac ttc gat tcg cac ccg gac 9408

Ser Phe Gly Cys Cys Ile Val Tyr Gln Asn Phe Asp Ser His Pro Asp

3120 3125 3130

agc cga gtc gaa gag cag cgg ctg cag atc ggg gtc ttg tcg cgg aac 9456

Ser Arg Val Glu Glu Gln Arg Leu Gln Ile Gly Val Leu Ser Arg Asn

3135 3140 3145

tac gag gct att aat gag ggg ctc gtg cat gat ctt gtt att gct ggg 9504

Tyr Glu Ala Ile Asn Glu Gly Leu Val His Asp Leu Val Ile Ala Gly

3150 3155 3160

gag tcg gag cct gat ggg gat gat ttg agg gtt act gtt gtg gcg aat 9552

Glu Ser Glu Pro Asp Gly Asp Asp Leu Arg Val Thr Val Val Ala Asn

3165 3170 3175 3180

cgg agg ttg tgc gat gag gaa agg ttg aag agg atg ctg gag gag ctg 9600

Arg Arg Leu Cys Asp Glu Glu Arg Leu Lys Arg Met Leu Glu Glu Leu

3185 3190 3195

tgt ggg aat att cgg gct ttg gcg ttg gtt tag 9633

Cys Gly Asn Ile Arg Ala Leu Ala Leu Val

3200 3205

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<212> PRT

<213> Mycelia sterilia

<400> 2

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Leu Ser Leu Ser Cys Pro Leu Leu Pro His Asp Asp Glu Lys His Ser

20 25 30

Asp Asn Leu Tyr Glu Gln Ala Thr Arg His Phe Gly Leu Ser Arg Asp

35 40 45

Lys Ile Glu Asn Val Leu Pro Cys Thr Ser Phe Gln Cys Asp Val Ile

50 55 60

Asp Cys Ala Val Asp Asp Arg Arg His Ala Ile Gly His Val Val Tyr

65 70 75 80

Asp Ile Pro Asn Thr Val Asp Ile Gln Arg Leu Ala Ala Ala Trp Lys

85 90 95

Glu Val Val Arg Gln Thr Pro Ile Leu Arg Thr Gly Ile Phe Thr Ser

100 105 110

Glu Thr Gly Asp Ser Phe Gln Ile Val Leu Lys Glu Gly Cys Leu Pro

115 120 125

Trp Met Tyr Ala Thr Cys Leu Gly Met Lys Gly Ala Val Ile Gln Asp

130 135 140

Glu Ala Val Ala Ala Met Thr Gly Pro Arg Cys Asn Arg Tyr Val Val

145 150 155 160

Leu Glu Asp Pro Ser Thr Lys Gln Arg Leu Leu Ile Trp Thr Phe Ser

165 170 175

His Ala Leu Val Asp Tyr Thr Val Gln Glu Arg Ile Leu Gln Arg Val

180 185 190

Leu Thr Val Tyr Asp Gly Arg Asp Val Glu Cys Pro Arg Ile Lys Asp

195 200 205

Thr Glu His Val Ser Arg Phe Trp Gln Gln His Phe Glu Gly Leu Asp

210 215 220

Ala Ser Val Phe Pro Leu Leu Pro Ser His Leu Thr Val Cys Asn Pro

225 230 235 240

Asn Ala Arg Ala Glu His His Ile Ser Tyr Thr Gly Pro Val Gln Arg

245 250 255

Lys Trp Ser His Thr Ser Ile Cys Arg Ala Ala Leu Ala Val Leu Leu

260 265 270

Ser Arg Phe Thr His Ser Ser Glu Ala Leu Phe Gly Val Val Thr Glu

275 280 285

Gln Ser His Asn Ser Glu Asp Gln Arg Arg Ser Ile Asp Gly Pro Ala

290 295 300

Arg Thr Val Val Pro Ile Arg Val Leu Cys Ala Pro Asp Gln Tyr Val

305 310 315 320

Ser Asp Val Ile Gly Ala Ile Thr Ala His Glu His Ala Met Arg Gly

325 330 335

Phe Glu His Ala Gly Leu Arg Asn Ile Arg Arg Thr Gly Asp Asp Gly

340 345 350

Ser Ala Ala Cys Gly Phe Gln Thr Val Leu Leu Val Thr Asp Gly Asp

355 360 365

Ala Pro Lys Thr Pro Gly Ser Val Leu His Arg Ser Val Glu Glu Ser

370 375 380

Asp Arg Phe Met Pro Cys Ala Asn Arg Ala Leu Leu Leu Asp Cys Gln

385 390 395 400

Met Ala Gly Asn Ser Ala Ser Leu Val Ala Arg Tyr Asp His Asn Val

405 410 415

Ile Asp Pro Arg Gln Met Ser Arg Phe Leu Arg Gln Leu Gly Tyr Leu

420 425 430

Ile Gln Gln Phe His His His Val Asp Leu Pro Leu Val Lys Glu Leu

435 440 445

Asp Val Val Thr Ala Glu Asp Cys Ala Glu Ile Glu Lys Trp Asn Ser

450 455 460

Glu Arg Leu Thr Met Gln Asp Ala Leu Ile His Asp Thr Ile Ser Lys

465 470 475 480

Trp Ala Ala Gly Asp Pro Asn Lys Ala Ala Val Phe Ala Trp Asp Gly

485 490 495

Glu Trp Thr Tyr Ala Glu Leu Asp Asn Ile Ser Ser Arg Leu Ala Val

500 505 510

Tyr Ile Gln Ser Leu Asp Leu Arg Pro Gly Gln Ala Ile Leu Pro Leu

515 520 525

Cys Phe Glu Lys Ser Lys Trp Val Val Ala Thr Ile Leu Ala Val Leu

530 535 540

Lys Val Gly Arg Ala Phe Thr Leu Ile Asp Pro Cys Asp Pro Ser Ala

545 550 555 560

Arg Met Ala Gln Val Cys Gln Gln Thr Ser Ala Thr Val Ala Leu Thr

565 570 575

Ser Lys Leu His Asn Thr Thr Leu Arg Ser Val Val Ser Arg Cys Ile

580 585 590

Val Val Asp Asp Asp Leu Leu Arg Ser Leu Pro His Ala Asp Gly Arg

595 600 605

Leu Lys Ala Thr Val Lys Pro Gln Asp Leu Ala Tyr Val Ile Phe Thr

610 615 620

Ser Gly Ser Thr Gly Glu Pro Lys Gly Ile Met Ile Glu His Arg Gly

625 630 635 640

Phe Val Ser Cys Ala Met Lys Phe Gly Pro Ala Leu Gly Met Asp Glu

645 650 655

His Thr Arg Ala Leu Gln Phe Ala Ser Tyr Ala Phe Gly Ala Cys Leu

660 665 670

Val Glu Val Val Thr Ala Leu Met His Gly Gly Cys Val Cys Ile Pro

675 680 685

Ser Asp Asp Asp Arg Leu Asn Asn Val Pro Glu Phe Ile Lys Arg Ala

690 695 700

Gln Val Asn Trp Val Ile Leu Thr Pro Ser Tyr Ile Gly Thr Phe Gln

705 710 715 720

Pro Glu Asp Val Pro Gly Leu Gln Thr Leu Val Leu Val Gly Glu Pro

725 730 735

Ile Ser Ala Ser Ile Arg Asp Thr Trp Ala Ser Gln Val Arg Leu Leu

740 745 750

Asn Ala Tyr Gly Gln Ser Glu Ser Ser Thr Met Cys Ser Val Thr Glu

755 760 765

Val Ser Pro Leu Ser Leu Glu Pro Asn Asn Ile Gly Arg Ala Val Gly

770 775 780

Ala Arg Ser Trp Ile Ile Asp Pro Asp Glu Pro Asp Arg Leu Ala Pro

785 790 795 800

Ile Gly Cys Ile Gly Glu Leu Val Ile Glu Ser Pro Gly Ile Ala Arg

805 810 815

Asp Tyr Ile Ile Ala Pro Pro Pro Asp Lys Ser Pro Phe Leu Leu Ala

820 825 830

Pro Pro Ala Trp Tyr Pro Ala Gly Lys Leu Ser Asn Ala Phe Lys Phe

835 840 845

Tyr Lys Thr Gly Asp Leu Val Arg Tyr Gly Pro Asp Gly Thr Ile Val

850 855 860

Cys Leu Gly Arg Lys Asp Ser Gln Val Lys Ile Arg Gly Gln Arg Val

865 870 875 880

Glu Ile Ser Ala Val Glu Ala Ser Leu Arg Arg Gln Leu Pro Ser Asp

885 890 895

Ile Met Pro Val Ala Glu Ala Ile Lys Arg Ser Asp Ser Ser Gly Ser

900 905 910

Thr Val Leu Thr Ala Phe Leu Ile Gly Ser Ser Lys Ser Gly Asp Gly

915 920 925

Asn Gly His Ala Leu Ser Ala Ala Asp Ala Val Ile Leu Asp His Gly

930 935 940

Ala Thr Asn Glu Ile Asn Ala Lys Leu Gln Gln Ile Leu Pro Gln His

945 950 955 960

Ser Val Pro Ser Tyr Tyr Ile His Met Glu Asn Leu Pro Arg Thr Ala

965 970 975

Thr Gly Lys Ala Asp Arg Lys Met Leu Arg Ser Ile Ala Ser Lys Leu

980 985 990

Leu Gly Glu Leu Ser Gln Asn Val Thr Ser Gln Pro Ile Glu Lys His

995 1000 1005

Asp Ala Pro Ala Thr Gly Ile Glu Val Lys Leu Lys Glu Leu Trp Phe

1010 1015 1020

Leu Ser Leu Asn Leu Asn Pro Asn Ser Gln Asp Val Gly Ala Ser Phe

025 1030 1035 1040

Phe Asp Leu Gly Gly Asn Ser Ile Ile Ala Ile Lys Met Val Asn Met

1045 1050 1055

Ala Arg Ser Ala Gly Ile Ala Leu Lys Val Ser Asp Ile Phe Gln Asn

1060 1065 1070

Pro Thr Leu Ala Gly Leu Val Asp Val Ile Gly Arg Asp Pro Ala Pro

1075 1080 1085

Tyr Asn Leu Ile Pro Thr Thr Ala Tyr Ser Gly Pro Val Glu Gln Ser

1090 1095 1100

Phe Ala Gln Gly Arg Leu Trp Phe Leu Asp Gln Ile Glu Leu Asp Ala

105 1110 1115 1120

Leu Trp Tyr Leu Leu Pro Tyr Ala Val Arg Met Arg Gly Pro Leu His

1125 1130 1135

Ile Asp Ala Leu Thr Ile Ala Leu Leu Ala Ile Gln Gln Arg His Glu

1140 1145 1150

Thr Leu Arg Thr Thr Phe Glu Glu Gln Asp Gly Val Gly Val Gln Val

1155 1160 1165

Val His Ala Ser Pro Ile Ser Asp Leu Arg Ile Ile Asp Val Ser Gly

1170 1175 1180

Asp Arg Asn Ser Asp Tyr Leu Gln Leu Leu His Gln Glu Gln Thr Thr

185 1190 1195 1200

Pro Phe Ile Leu Ala Cys Gln Ala Gly Trp Arg Val Ser Leu Ile Arg

1205 1210 1215

Leu Gly Glu Asp Asp His Ile Leu Ser Ile Val Met His His Ile Ile

1220 1225 1230

Ser Asp Gly Trp Ser Ile Asp Ile Leu Arg Arg Glu Leu Ser Asn Phe

1235 1240 1245

Tyr Ser Ala Ala Leu Arg Gly Ser Asp Pro Leu Ser Val Val Ser Pro

1250 1255 1260

Leu Pro Leu His Tyr Arg Asp Phe Ser Val Trp Gln Lys Gln Val Glu

265 1270 1275 1280

Gln Glu Thr Glu His Glu Arg Gln Leu Glu Tyr Trp Val Lys Gln Leu

1285 1290 1295

Ala Asp Ser Ser Ala Ala Glu Phe Leu Thr Asp Phe Pro Arg Pro Asn

1300 1305 1310

Ile Leu Ser Gly Glu Ala Gly Ser Val Pro Val Thr Ile Glu Gly Glu

1315 1320 1325

Leu Tyr Glu Arg Leu Gln Glu Phe Cys Lys Val Glu Gln Met Thr Pro

1330 1335 1340

Phe Ala Val Leu Leu Gly Ala Phe Arg Ala Thr His Tyr Arg Leu Thr

345 1350 1355 1360

Gly Ala Glu Asp Ser Ile Ile Gly Thr Pro Ile Ala Asn Arg Asn Arg

1365 1370 1375

Gln Glu Leu Glu Asn Met Ile Gly Phe Phe Val Asn Thr Gln Cys Met

1380 1385 1390

Arg Ile Thr Val Asp Gly Asp Asp Thr Phe Glu Ser Leu Val Arg Gln

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Val Arg Thr Thr Ala Thr Ala Ala Phe Glu His Gln Asp Val Pro Phe

1410 1415 1420

Glu Arg Val Val Thr Ala Leu Leu Pro Arg Ser Arg Asp Leu Ser Arg

425 1430 1435 1440

Asn Pro Leu Ala Gln Leu Thr Phe Ala Leu His Ser Gln Gln Asp Leu

1445 1450 1455

Gly Lys Phe Glu Leu Glu Gly Leu Val Ala Glu Pro Val Ser Asn Lys

1460 1465 1470

Val Tyr Thr Arg Phe Asp Val Glu Phe His Leu Phe Gln Glu Ala Gly

1475 1480 1485

Arg Leu Ser Gly Asn Val Ala Phe Ala Ala Asp Leu Phe Lys Pro Glu

1490 1495 1500

Thr Ile Ser Asn Val Val Ala Ile Phe Phe Gln Ile Leu Arg Gln Gly

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Ile Arg Gln Pro Arg Thr Pro Ile Ala Val Leu Pro Leu Thr Asp Gly

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Leu Ala Asp Leu Arg Ala Met Gly Leu Leu Glu Ile Glu Lys Ala Glu

1540 1545 1550

Tyr Pro Arg Glu Ser Ser Val Val Asp Val Phe Arg Lys Gln Val Ala

1555 1560 1565

Ala His Pro His Ala Phe Ala Val Val Asp Ser Ala Ser Arg Leu Thr

1570 1575 1580

Tyr Ala Asp Leu Asp Arg Gln Ser Asp Gln Leu Ala Thr Trp Leu Gly

585 1590 1595 1600

Arg Arg Asn Met Thr Ala Glu Thr Leu Val Gly Val Leu Ala Pro Arg

1605 1610 1615

Ser Cys Gln Thr Val Val Ala Ile Leu Gly Ile Leu Lys Ala Asn Leu

1620 1625 1630

Ala Tyr Leu Pro Leu Asp Val Asn Cys Pro Thr Ala Arg Leu Gln Thr

1635 1640 1645

Ile Leu Ser Thr Leu Asn Arg His Lys Leu Val Leu Leu Gly Ser Asn

1650 1655 1660

Ala Thr Thr Pro Asp Val Gln Ile Pro Asp Val Glu Leu Val Arg Ile

665 1670 1675 1680

Ser Asp Ile Leu Asp Arg Pro Ile Asn Gly Gln Ala Lys Leu Asn Gly

1685 1690 1695

His Thr Lys Ser Asn Gly Tyr Ser Lys Pro Asn Gly Tyr Thr His Leu

1700 1705 1710

Lys Gly Tyr Ser Asn Leu Asn Gly Tyr Ser Lys Gln Asn Gly Tyr Ala

1715 1720 1725

Gln Leu Asn Gly His Arg Glu Arg Asn Asn Tyr Leu Asp Leu Asn Gly

1730 1735 1740

His Ser Leu Leu Asn Gly Asn Ser Asp Ile Thr Thr Ser Gly Pro Ser

745 1750 1755 1760

Ala Thr Ser Leu Ala Tyr Val Ile Phe Thr Ser Gly Ser Thr Gly Lys

1765 1770 1775

Pro Lys Gly Val Met Val Glu His Arg Ser Ile Ile Arg Leu Ala Lys

1780 1785 1790

Lys Asn Arg Ile Ile Ser Arg Phe Pro Ser Val Ala Lys Val Ala His

1795 1800 1805

Leu Ser Asn Ile Ala Phe Asp Ala Ala Thr Trp Glu Met Phe Ala Ala

1810 1815 1820

Leu Leu Asn Gly Gly Thr Leu Val Cys Ile Asp Tyr Met Thr Thr Leu

825 1830 1835 1840

Asp Ser Lys Thr Leu Glu Ala Ala Phe Ala Arg Glu Gln Ile Asn Ala

1845 1850 1855

Ala Leu Leu Thr Pro Ala Leu Leu Lys Gln Cys Leu Ala Asn Ile Pro

1860 1865 1870

Thr Thr Leu Gly Arg Leu Ser Ala Leu Val Ile Gly Gly Asp Arg Leu

1875 1880 1885

Asp Gly Gln Asp Ala Ile Ala Ala His Ala Leu Val Gly Ala Gly Val

1890 1895 1900

Tyr Asn Ala Tyr Gly Pro Thr Glu Asn Gly Val Ile Ser Thr Ile Tyr

905 1910 1915 1920

Asn Ile Thr Lys Asn Asp Ser Phe Ile Asn Gly Val Pro Ile Gly Cys

1925 1930 1935

Ala Ile Ser Asn Ser Gly Ala Tyr Ile Thr Asp Pro Asp Gln Gln Leu

1940 1945 1950

Val Pro Pro Gly Val Met Gly Glu Leu Val Val Thr Gly Asp Gly Leu

1955 1960 1965

Ala Arg Gly Tyr Thr Asp Pro Ala Leu Asp Ala Gly Arg Phe Val Gln

1970 1975 1980

Ile Met Ile Asn Asp Lys Ala Val Arg Ala Tyr Arg Thr Gly Asp Arg

985 1990 1995 2000

Ala Arg Tyr Arg Val Gly Asp Gly Gln Ile Glu Phe Phe Gly Arg Met

2005 2010 2015

Asp Gln Gln Val Lys Ile Arg Gly His Arg Ile Glu Pro Ala Glu Val

2020 2025 2030

Glu Arg Ala Ile Leu Asp Gln Asp Ser Ala Arg Asp Ala Val Val Val

2035 2040 2045

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2050 2055 2060

Thr His Gly Asp His Ser Ala Glu Gln Glu Glu Ala Asp Asp Gln Val

065 2070 2075 2080

Glu Gly Trp Lys Asp Phe Phe Glu Ser Asn Thr Tyr Ala Asp Met Asp

2085 2090 2095

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Asp Asp Thr Met Arg Thr Leu Leu Asp Gly Gln Ala Pro Gly His Val

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Leu Glu Ile Gly Thr Gly Ser Gly Met Val Leu Phe Asn Leu Gly Ala

145 2150 2155 2160

Gly Leu Gln Ser Tyr Val Gly Leu Glu Pro Ser Arg Ser Ala Ala Thr

2165 2170 2175

Phe Val Thr Lys Ala Ile Asn Ser Thr Pro Ala Leu Ala Gly Lys Ala

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Glu Val His Val Gly Thr Ala Thr Asp Ile Asn Arg Leu Arg Gly Leu

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225 2230 2235 2240

Val Lys Arg Val Val Phe Gly Asp Ile Arg Ser His Ala Thr Asn Arg

2245 2250 2255

His Phe Leu Ala Ala Arg Ala Leu His Ser Leu Gly Ser Lys Ala Thr

2260 2265 2270

Lys Asp Ala Ile Arg Gln Lys Met Thr Glu Met Glu Glu Arg Glu Glu

2275 2280 2285

Glu Leu Leu Val Asp Pro Ala Phe Phe Thr Ala Leu Leu Gln Gly Gln

2290 2295 2300

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Ala Thr Asn Glu Leu Ser Ala Tyr Arg Tyr Thr Ala Val Ile His Val

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Arg Gly Pro Glu Glu Gln Ser Arg Pro Val Tyr Pro Ile Gln Val Asn

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Asp Trp Ile Asp Phe Gln Ala Ser Arg Ile Asp Arg Arg Ala Leu Leu

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2370 2375 2380

Ile Pro Tyr Ser Lys Thr Ile Val Glu Arg His Val Val Glu Ser Leu

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Trp Ile Ser Ala Val Arg Ser Lys Ala Glu Arg Cys Thr Ser Leu Ser

2420 2425 2430

Val Thr Asp Leu Val Gln Leu Gly Glu Glu Ala Gly Phe Arg Val Glu

2435 2440 2445

Val Ser Ala Ala Arg Gln Trp Ser Gln Ser Gly Ala Leu Asp Ala Val

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Phe His Arg Tyr Asn Leu Pro Thr Gln Ser Asn Ser Arg Val Leu Ile

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Gln Phe Pro Thr Glu Asp Gly Gln Thr Arg Arg Ser Ala Thr Leu Thr

2485 2490 2495

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Ile Val Val Ile Asp Gln Met Pro Leu Asn Ala Asn Gly Lys Val Asp

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Arg Lys Glu Leu Thr Arg Arg Ala Gln Ile Ala Pro Lys Ser Gln Ala

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Ala Pro Ala Lys Pro Val Lys Gln Val Asp Pro Phe Val Asn Leu Glu

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Val Asn Asp His Phe Phe Gln Leu Gly Gly His Ser Leu Leu Ala Thr

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Arg Gln Gly Leu Thr Leu Glu Asn Ala Ile Pro Ala Thr Pro Asp Ser

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Gly Tyr Trp Glu Gln Thr Met Ser Ala Pro Thr Thr Pro Ser Asp Asp

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Met Glu Ala Val Leu Cys Lys Glu Phe Ala Asp Val Leu Gly Val Glu

2675 2680 2685

Val Ser Ala Thr Asp Ser Phe Phe Asp Leu Gly Gly His Ser Leu Met

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Ala Thr Lys Leu Ala Ala Arg Ile Ser Arg Arg Leu Asp Val Pro Val

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Ser Ile Lys Asp Ile Phe Asp His Ser Val Pro Leu Asn Leu Ala Arg

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Gln Ile Ala Asn Asp Ala Pro Phe Gln Leu Ile Ser Val Glu Asp Pro

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Leu Leu Asn Pro Val Thr Gly Lys Pro Arg Ser Pro Thr Pro Phe His

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Ile Asp Phe Pro Pro Asp Ala Asp Cys Ala Ser Leu Met Arg Ala Cys

2820 2825 2830

Ala Ser Leu Ala Lys His Phe Asp Ile Phe Arg Thr Val Phe Leu Glu

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Ala Arg Gly Glu Leu Tyr Gln Val Val Leu Lys His Val Asp Val Pro

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Ile Glu Met Leu Gln Thr Glu Glu Asn Ile Asn Ser Ala Thr Arg Ser

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Phe Leu Asp Val Asp Ala Glu Lys Pro Ile Arg Leu Gly Gln Pro Leu

2885 2890 2895

Ile Arg Ile Ala Ile Leu Glu Lys Pro Gly Ser Thr Leu Arg Val Ile

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Leu His Ser Leu His Ile Leu Phe Phe Gly Gly Ser Leu Pro Pro Pro

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Pro Lys Phe Ala Gly Tyr Met Gln His Val Ala Ser Ser Arg Arg Glu

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Gly Tyr Asp Phe Trp Arg Ser Val Leu Arg Asp Ser Ser Met Thr Val

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2995 3000 3005

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Gln Glu Gln Tyr Leu Asn Ser Leu Ala Phe Glu Thr Leu Gly Tyr Asp

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Glu Ile Lys Ala His Cys Thr Asp Trp Pro Asp Val Pro Ala Thr Ala

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Ser Phe Gly Cys Cys Ile Val Tyr Gln Asn Phe Asp Ser His Pro Asp

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Ser Arg Val Glu Glu Gln Arg Leu Gln Ile Gly Val Leu Ser Arg Asn

3140 3145 3150

Tyr Glu Ala Ile Asn Glu Gly Leu Val His Asp Leu Val Ile Ala Gly

3155 3160 3165

Glu Ser Glu Pro Asp Gly Asp Asp Leu Arg Val Thr Val Val Ala Asn

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Cys Gly Asn Ile Arg Ala Leu Ala Leu Val

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agcatcggat cctaacaatg tcaaacatgg caccactccc ta 42

[Brief description of the drawings]

[Figure 1]

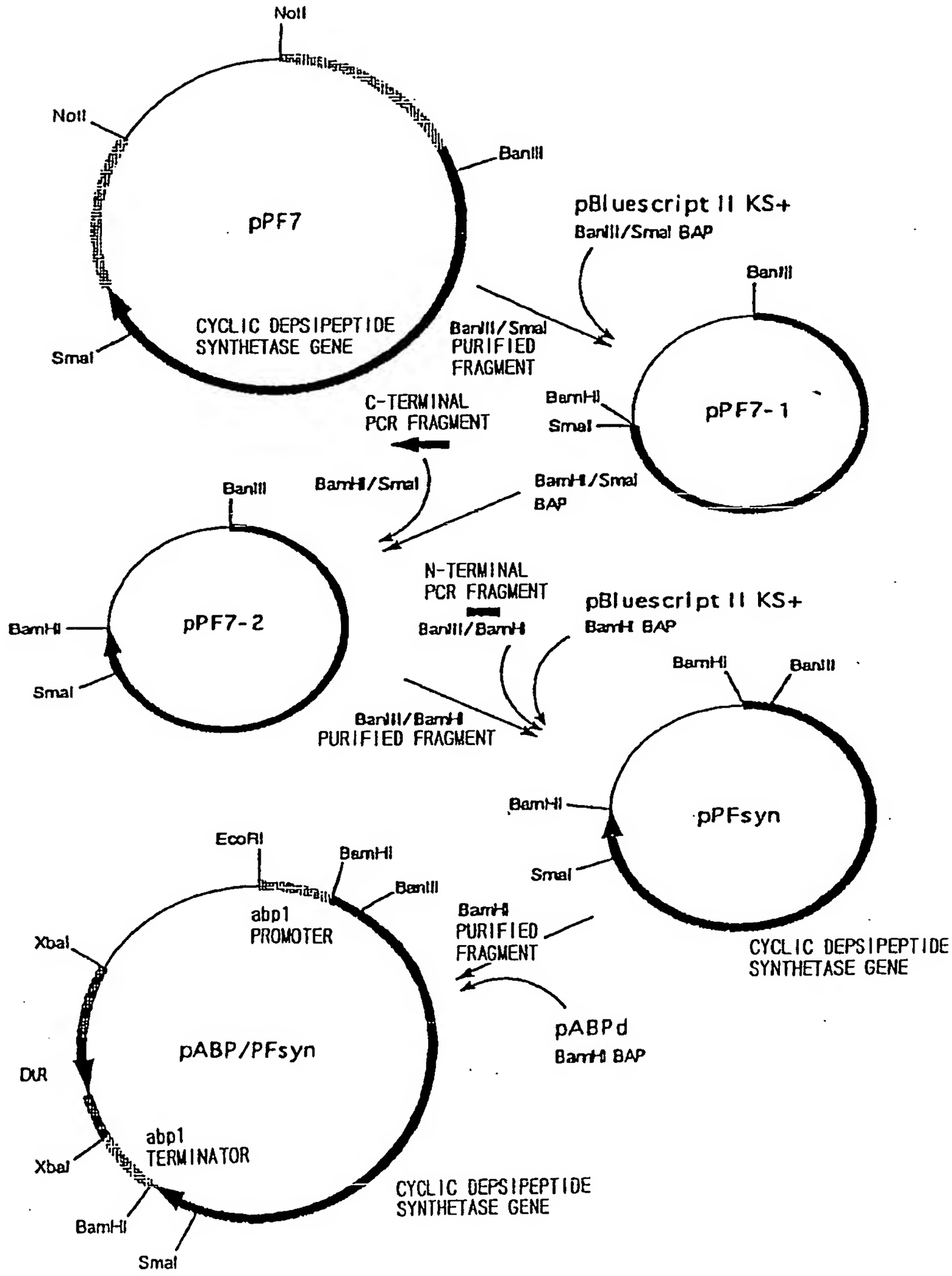
Figure 1 shows a construction procedure of plasmid pABP/PFsyn.

[Figure 2]

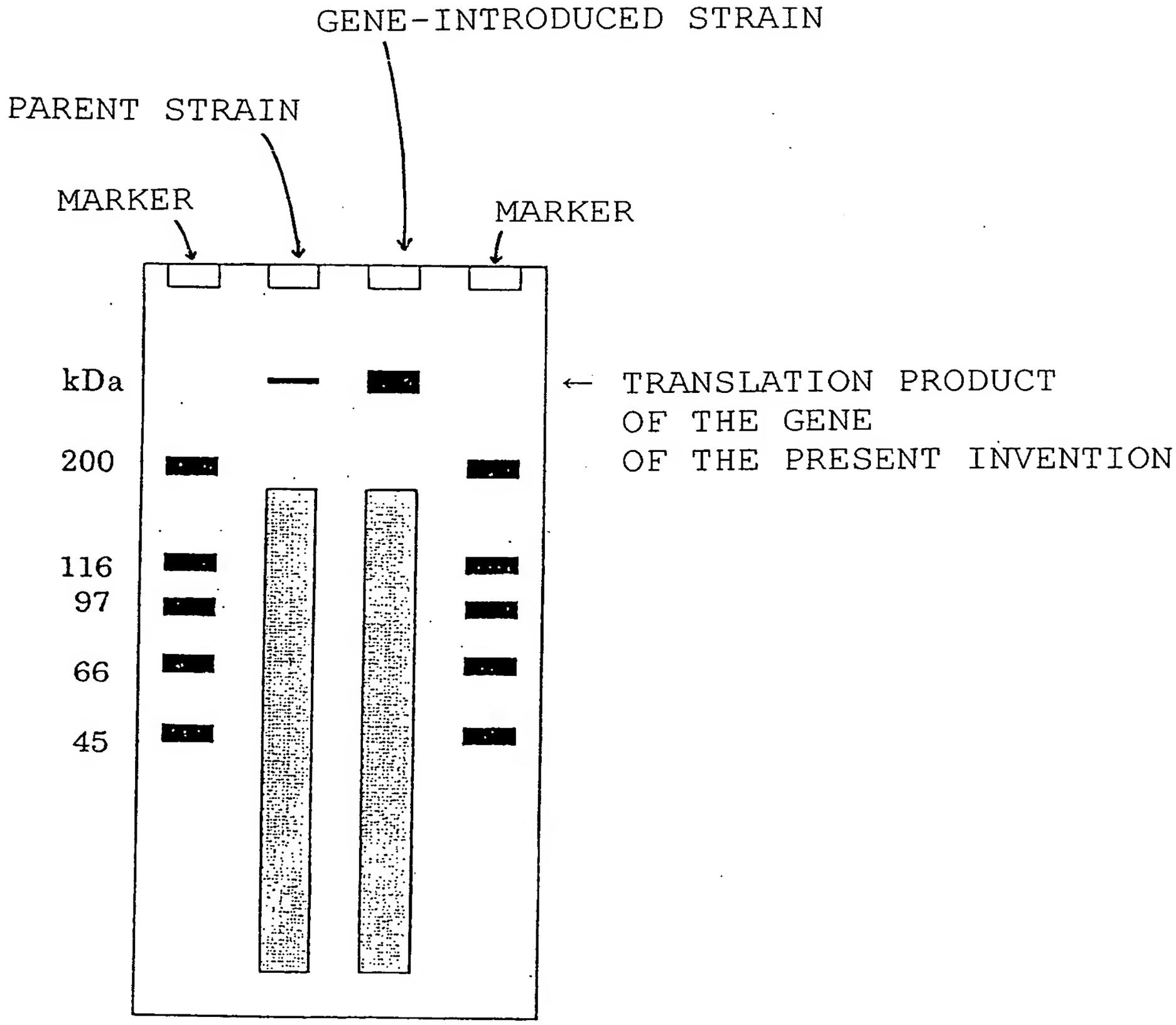
Figure 2 schematically shows the SDS-PAGE result of the expression, in a gene-introduced strain, of the cyclic depsipeptide synthetase which is the translation product of the gene of the present invention.

[TITLE OF DOCUMENT] DRAWINGS 11-253040

[Figure 1]



[Figure 2]


GENE-INTRODUCED STRAIN
PARENT STRAIN
MARKER
MARKER
kDa
200
116
97
66
45
← TRANSLATION PRODUCT
OF THE GENE
OF THE PRESENT INVENTION

[TTTLE OF DOCUMENT] ABSTRACT

[ABSTRACT]

[OBJECT OF INVENTION]

To provide a method for producing a cyclic depsipeptide having anthelmintic activity, a cyclic depsipeptide synthetase gene capable of improving productivity of the substance PF1022 and a protein coding therefor, a recombinant vector comprising the gene, and a substance PF1022-producing microorganism and substance PF1022 into which the recombinant vector is introduced.

[MEANS FOR ATTAINING THE OBJECT]

A gene encoding a cyclic depsipeptide synthetase enhancing the biosynthesis of the substance PF1022 is isolated from a substance PF1022-producing microorganism. A recombinant vector is prepared by the substitution of the promoter and terminator of the gene with those of a foreign gene utilizable for expression augmentation, and then the recombinant vector is introduced into the substance PF 1022-producing microorganism, whereby the improvement in productivity of the substance PF 1022 was attained.

[SELECTED FIGURE]

None